



Unit Three Lesson: THE MICROENVIRONMENT, ITS ROLE IN REGULATING STEM CELL FATE, CANCER

Darci Kimball, Laurel Barchas, Doug Brownfield

California State Standards:

Biology/Life Science

1.a. Students know cells are enclosed within semi-permeable membranes that regulate their interaction with their surroundings.

4. Genes are a set of instructions encoded in the DNA sequence of each organism that specify the sequence of amino acids in proteins characteristic of that organism. As a basis for understanding this concept:

4a. Students know the general pathway by which ribosomes synthesize proteins, using tRNAs to translate genetic information in mRNA.

“After learning about transcription and translation through careful study of expository texts, students can simulate the processes on paper or with representative models.”

4b. Students know how to apply the genetic coding rules to predict the sequence of amino acids from a sequence of codons in RNA. “In eukaryotes, the initial RNA transcript, while in the nucleus, is composed of exons, sequences of nucleotides that carry useful information for protein synthesis, and introns, sequences that do not. Before leaving the nucleus, the initial transcript is processed to remove introns and splice exons together. The processed transcript, then properly called mRNA and carrying the appropriate codon sequence for a protein, is transported from the nucleus to the ribosome for translation.”

4d. Students know specialization of cells in multicellular organisms is usually due to different patterns of gene expression rather than to differences of the genes themselves. “Nearly all cells in an organism contain the same DNA, but each cell transcribes only that portion of DNA containing the genetic information for proteins required at that specific time by that specific cell. The remainder of the DNA is not expressed. Specific types of cells may produce specific proteins unique to that type of cell.”

5.b. Students know how to apply base-pairing rules to explain precise copying of DNA during semiconservative replication and transcription of information from DNA into mRNA.

**Goals:**

- Understand that genes are a set of instructions, encoded by the DNA sequence, that denote the types and sequence of amino acids during protein synthesis.
- Demonstrate the central dogma of biology by modeling the transcription of a DNA sequence to mRNA and the translation of mRNA codon triplets into amino acids that fold to form a protein.
- Explain the significance of signals from the microenvironment and gene expression in the formation of specialized cells in multi-cellular organisms.

Objectives:

1. Students will be able to define *genotype*, *phenotype* and *microenvironment* in terms of cell fate decision and how a cell is able to specialize appropriately.
2. Students will be able to differentiate between genotype and gene expression in terms of protein synthesis (which results in a phenotype).
3. Students will be able to explain the relationship between DNA, mRNA, and tRNA as well as the processes used to form a protein during protein synthesis.
4. Students will be able to simulate the production of different types of mature cells from an adult stem cell depending on signals from the microenvironment using the framework of the central dogma of biology. AP students can begin to understand the limitations of the central dogma, in that RNA often regulates gene expression.
5. Students will be able to recognize factors in the microenvironment which control gene expression and cell fate, including signaling proteins, extracellular matrix proteins, forces, and cell-to-cell interactions.
6. Students will be able to describe how factors in the microenvironment signal stem cells to proliferate or differentiate (example mammary gland progenitor cell).
7. Students will be able to investigate applications of appropriate cell response to signaling in topics such as colon stem cells and hair follicles while exploring how aberrant gene expression could *but does not always* lead to cancer.
8. Students will be able to simulate a reductionist method to understanding the role of the microenvironment by simulating array systems and by modeling a Microenvironment Array.



Outline of Unit Three

I. Invitation

A. Show animation: Colony Stimulating Factor (Drew Berry and Etsuko Uno, 2009)
Scroll to bottom to find CSF animation.

[Download animation here](#)

Synopsis of animation: When the immune system recognizes a pathogenic invasion, immune cells respond by releasing colony stimulating factors (CSF), which stimulate stem cells in the bone marrow to construct specialized white blood cells. These white blood cells begin to form a colony, which will continue to undergo cell division. As the colony grows and matures, the formation of an immune cell army then travels through the circulatory system with a mission to eradicate the infection. Since their discovery, colony stimulating factors have helped millions of cancer patients survive destruction to the bone marrow that often results from high-dose chemotherapy.

B. For an introduction or review of the genetic processes underlying the above proliferation, show these animations on transcription and translation and ask the student WHY these processes are important to the effects of Colony Stimulating Factor on bone marrow stem cells.

1. [Navigate to translation and transcription animations](#) then scroll down to DNA Central Dogma Part 1 and Part 2 animations displayed on the page. These can be played through your browser window by clicking play or can be downloaded individually by clicking "Download the Animation"

a. DNA Central Dogma Part 1 Transcription (Drew Berry, 2003)

b. DNA Central Dogma Part 2 Translation (Drew Berry, 2003)

2. Have students practice transcription and translation using the following interactive animation. [Download here](#)



C. AP Extension Activity: How does a cell with one genotype give rise to cells with different phenotypes?

1. Choices of a Common Myeloid Progenitor cell
 - a. Become erythrocytes (red blood cells)
 - b. Become granulocytes (white blood cells)
2. Use “From Genotype to Phenotypes: Red Blood Cell or White Blood Cell?” handout for the following exercises (Appendix A) [From Genotype to Phenotypes](#) and “[Instructions for Microenvironment Array Activity](#)”. This activity requires preparation in advance.
3. In this exercise, your students will simulate the production of EITHER red blood cells OR white blood cells from common myeloid progenitor cells. After receiving instruction from the teacher to use either soluble factor signaling molecules) a) Erythropoietin OR b) Granulocyte-Colony Stimulating Factor, students will go through the process of transcription and translation to create either a) RED protein or b) WHITE protein.
4. Download Another Plasmid Editor (ApE) for teacher and all student computers. Scroll down for Windows or MAC version [Download here](#)

II. Exploration

A. *Cell fate and behavior*

1. Lecture and Discussion-How does cell phenotype come from genotype?
 - a. We have 30,000 genes, but only a subset are expressed for each cell type
 - b. Through gene expression, transcription and translation of this subset of genes determines how a cell looks and acts (its phenotype)
 - i. Phenotype: Proteins and enzymes, surface markers, cell behaviors (migration), cell fate decisions (proliferation and differentiation).
 - ii. What dictates potential? Pluripotent and Multipotent SCs respond differently to signals because they express different genes. These characteristics are results of the differential expression of subsets of genes.
 - c. How does a cell know what to become? In a cell fate decision, a stem cell makes a decision to behave, for example proliferate, differentiate, or migrate. Is this decision random? If not, what influences this decision?



(The decisions to proliferate and differentiate don't have to be mutually exclusive. For example, Granulocyte-Colony Stimulating Factor influences *proliferation and then differentiation*)

- d. Central Dogma of Biology worksheets and “signaling” factors
 - i. Students can read about examples of microenvironmental factors and regulation below in 2.
 - ii. Use Central Dogma Handout (Appendix B), [Download handout](#)

2. Readings-Jigsaw activity. See general [instructions for jigsaw activity](#) in Unit One Appendix E. Pick several of these readings for students to read and understand the effects of each microenvironment component. Choose one from each of the sections in **bold**. Ask students to consider: What controls gene expression and cell fate? The microenvironment, which consists of: signaling factors (soluble like Colony Stimulating Factor and steroid hormones), extracellular matrix proteins, forces, and cell-cell interactions.

a. Signaling factors

- i. EASY: Protein receptor for neural cells [Download article](#)
- ii. EASY: Nerve growth factor: Easy level reading [Download article](#)
- iii. MID: Stat3 turns off immune system and allows cancer to form [Download article](#)
- iv. MID: Protein signaling neural formation and Notch pathways [Download article](#)
- v. HARD: Proteins that cause stem cells to form skin cells [Download article](#)

b. Extracellular matrix proteins

- i. EASY: ECM protein that promotes muscle cell health [Download article](#)
- ii. MID: ECM determines cell fate and cancer development [Download article](#)
- iii. MID: Cell movement regulated between cells [Download article](#)
- iv. MID: UCSD research summary of ECM role on cells [Download article](#)
- v. HARD: General overview on role of ECM [Download article](#)
- vi. HARD: Role of ECM in cell differentiation



[Download article](#)

c. Forces

- i. EASY/press release: [Download article](#)
- ii. MID: Affect of mechanical forces on embryonic stem cells
[Download article](#)
- iii. MID: Cell orientation among other cells determines its division
[Download article](#)

d. Cell-cell interactions

- i. MID: Hormone receptors inside cells
[Download article](#)
- ii. HARD: Inter-cell communication
[Download article](#)
- iii. HARD: Cell–cell interaction networks regulate cell fate
[Download article](#)

3. Lab exercise

For teachers near UC San Diego, you can use the RNAi and Genetics lab kit and protocol from BioBridge @ UCSD. RNAi is an excellent way to examine the process of the central dogma. In this lab, we will look at the microscopic nematode, *C. elegans*. These worms eat bacteria and you feed them bacteria that have been transformed with an RNAi molecule. This molecule degrades the production of collagen in the worms. Over three days time, this degradation of collagen will cause the worm to lose its shape and become short and fat, or "dumpy." This lab allows the students to make a visual connection between RNA, protein and phenotype. You can ask students: how did changing gene expression affect the microenvironment? What are the observable changes on a macro scale? What might happen to cells as a result of this drastically changed microenvironment? You must receive training on teaching this lab before picking up the kit. For more information, visit: [Download Biobridge kit and protocol](#)
Ignore web site notice if it comes up by clicking "ok".

4. AP extension Video observation and discussion

Cell behavior is similarly determined by signals from outside the cell. Example is cell migration (a property of stem cells as well as somatic cells such as fibroblasts.) What determines the movement of a cell?

- a. Cell invasions video: [Download here](#)



B. Microenvironment and Cancer

Cells live in tissue (connective tissue and other cells). The microenvironment around the cell is anything to which the cell could potentially respond, typically by proliferating or differentiating, or by exhibiting a behavior. It also may keep cancer cells “in check”.

1. Lecture using supplementary Microenvironment PowerPoint slides ([Unit Three Microenvironment PowerPoint](#)) and information below.

a. Two examples of normal microenvironments:

- i. View the animation Intestinal Crypt Stem Cells - A Clonal Conveyor Belt. By Eric Keller/Digizyme: [Clonal Conveyor Belt animation](#) (enter animation search “clonal conveyor belt” click View the animation) Preload, as this can take time to load.
- ii. Hair follicles- Examination and Web Exploration
Examine the cells in the hair follicle using pictures from this site: [Hair follicle pictures](#) Teacher can also use prepared slides of hair follicles. (Buy slides from Wards, Fisher, etc.)

b. Optional: Teach the different types of epithelial tissues and connective tissues using virtual labs. [Tissue Identification in virtual lab](#)

c. Discuss...what is the hair follicle microenvironment? Where are the stem cells housed and what are their microenvironments (what cells are next to them, etc.)? What might be the result of changing this particular microenvironment? Ex. Hormones (testosterone, other androgens) in or near the hair follicle increases growth of hair. Can you think of any products that increase hair growth and how they might work? (Students could search for this information online.)

2. Aberrant gene expression combined with abnormal microenvironment = Cancer: Lecture and discussion

- a. Cancer is caused by mutations in proto-oncogenes, resulting in oncogene expression (lecture from background information)
- b. There are different hypothesis to explain how cancer grows and spreads: examples are the Cancer Stem Cell hypothesis and the Clonal Evolution Model. Describe how the Cancer Stem Cell hypothesis differs from the Clonal Evolution Model. Refer to the diagram available here: [Download here](#) Click “Download PDF” to read the article; Figure 1 is a diagram visually comparing the two hypotheses.



3. Readings-Have students pick one of the following articles to read first.
 - a. EASY-MID: American Cancer Society article “Oncogenes and Tumor Suppressor Genes” [Download](#)
 - b. MID-HARD: Nature SciTable article “Gene Expression Regulates Cell Differentiation” [Download article](#)

Then, assign one or more of the following articles to students.

- a. EASY LEAD-IN - Science Daily Article and Video: Unraveling brain tumors [Download](#)
- b. EASY/MID – Researchers Find Prostate Cancer Stem Cell [Download](#)
- c. EASY (Abstract) - Dennis Disher, Tissue Cells Feel and Respond to the Stiffness of their Substrate. Science magazine article [Download](#)
- d. MID – The Stem Cell Hypothesis [Stem Cell Hypothesis article](#)
- e. MID/CHALLENGING – Stem Cells May Be Key to Cancer, NY Times article <http://www.nytimes.com/2006/02/21/health/21canc.html>
- f. (News Report) UCSD researchers pave the way for effective treatments [Download Liver Treatment article](#)
- g. MID – Diabetes Drug Selectively Kills Cancer Stem Cells In Combination Treatment in Mice [Download](#)
- h. MID (News Report) -- Sean Morrison challenges cancer stem cell existence in melanoma [Download](#)
- i. CHALLENGING (Abstract with figure) Mattais Lutolf @ Stanford – hematopoietic stem cell ME [Download](#)
- j. VERY CHALLENGING (the research paper from 7.) – Metformin Selectively Targets Cancer Stem Cells, and Acts Together with Chemotherapy to Block Tumor Growth and Prolong Remission [Download](#)

4. AP extension Discussion Question

Odd gene expression pattern causes cancer, but sometimes cells with these genetic mutations are not necessarily “cancerous,” i.e. they lack the phenotype of a cancer cell. What could be preventing them from doing so?



III. Application

A. Lecture-Use Teacher Background Information-Human Mammary Gland Progenitor Cells. Understand the role of the microenvironment in normal development and cancer (lecture from Background Information section).

B. Inspection and understanding of arrays *in general* by looking at cDNA array methods and data or other types of simpler array systems like Enzyme-linked Immunosorbent Assay (ELISA).

1. Dolan DNA Learning Center – cDNA array animation

[Download cDNA array animation](#)

OR

[DNA Microarray flash animation](#)

2. Microbiology, Prescott, Harley, and Klein — ELISA animation

[ELISA animation](#)

C. Understand immunocytochemistry using fluorescently-tagged antibodies.

1. Antibodies are an important research tool for cell biology. Scientists use antibodies generated in different animals to identify specific types of stem cells based on the different molecules those cells produce on their surfaces or inside their nuclei. To visualize a certain type of stem cell using fluorescence microscopy, a scientist would prepare a thin slice of tissue or a small dish of cells, then incubate the tissue or cells in what's called a "primary antibody" specific to one molecule on the surface of the stem cell of interest. This primary antibody recognizes only those molecules and attaches to them. Next, the scientist would incubate the tissue slice or cells in a "secondary antibody" fused to a fluorescent molecule. This secondary antibody only recognizes and binds to the primary antibody. Now, the scientist looks at it under a fluorescent microscope. The microscope uses a certain wavelength of light that excites the fluorescent molecules marking the stem cells of interest, causing them to emit another wavelength of light that can be detected by the microscope and your eye. See readings here:

a. EASIER - Immunofluorescence Labeling method

[Immunofluorescence labeling](#)

b. HARDER – Immunofluorescence Labeling of Cells, Sigma-Aldrich

[Immunofluorescence Labeling, Sigma-Aldrich](#)

c. Mammary gland picture from LaBarge lab

[Appendix C](#) [ppt]



d. Fluorescently-tagged stem cell pictures are available on CIRM's flickr page. [Fluorescently-tagged stem cell downloads](#)

D. Visualize “Breast Stem Cells”, “Control of Breast Stem Cells” and “Origin of Breast Cancer” by viewing animations at [Molecular movies](#). Type name of each video animation into site search window.

E. Understand and summarize either orally or with a short paper the Microenvironment Array (MEArray)

1. Mammary progenitor cell: turns into myoepithelial cells or luminal epithelial cells, but what are the signals telling them to change??

a. LBNL News Story: [Download LBNL News story here](#)

b. AP biology extension: Human mammary progenitor cell fate decisions are products of interactions with combinatorial microenvironments, read abstract and figure: [Download article](#)

F. Design your own MEArray research project: Help your students describe how a scientist would test putative ME factors' effects on mammary gland progenitors using MEArray technology. See MEArray Teacher Guide (Unit Three [Appendix D](#)) describing this project.

G. AP Extension questions: How do forces affect cell fate decisions? Can an ME Array test this? (No, it can only test ECM molecule- or signaling protein- interactions and cell-cell contact—not forces.) How would you test the effects of forces on stem or progenitor cells?

1. Short introduction: Growth factors, matrices, and forces combine and control stem cells. [Download article](#) (abstract)

2. Cell fate determination research covers three areas:

a. Adhesion-dependent cell survival

b. Biomechanical force and the control of cell phenotype

c. Adhesion-dependent manipulation of stem cell fate

3. For a MID/CHALLENGING overview of these areas: [Download](#)

4. See work of Sanjay Kumar's lab, UC Berkeley Bioengineering Department [Download](#)



5. Articles about how forces affect cell fate

- a. EASY/press release: [Download press releas](#)
- b. MID: [Download](#)
- c. MID/CHALLENGING: [Download Kumarlab article](#)
- d. MID/CHALLENGING: [Download Kumarlab article](#)
- e. VERY CHALLENGING: [Download Kumarlab article](#)

then scroll down and click the PDF link after this 2010 article:

J. Keung, K. E. Healy, S. Kumar, and D. V. Schaffer (2009). Biophysics and dynamics of natural and engineered stem cell microenvironments.

Wiley Interdisciplinary Reviews: Systems Biology and Medicine.

IV. Assessment

A. Have students answer as essay questions or compose exam to test understanding of topics

1. Describe how a cell's *genotype* is converted into its *phenotype*.
2. What are the four components of the *microenvironment*?
3. What is cell behavior? Give three examples.
4. Give a specific example of how the microenvironment guides a cell fate decision.
5. Put the following terms in their proper sequence of appearance during gene expression: protein, splicing, mRNA, DNA, translation, pre-RNA, transcription, tRNA.
6. Define a stem cell niche and give one example. (Intestinal crypt or hair follicle)
7. Describe in general how a Microenvironment Array/cDNA array/ELISA works and why a scientist would want to use it.

B. AP thought questions (possible web research project to answer these)

1. Is a genetic mutation necessary for cancer?
2. Could cancer occur only by manipulating the microenvironment?
3. If you took a piece of normal tissue and inserted it inside a tumor, what would happen?
4. If you infect a chicken embryo with a cancer-causing virus, and the chicken grew up cancer-free, would you assume the chicken's cells were noncancerous?
5. How might we test ways to see if certain microenvironments can stop cancer from growing?

**Resources:**

Science Daily Video: Unraveling brain tumors (COPYRIGHT *American Institute of Physics*)

http://www.sciencedaily.com/videos/2007/0910-unraveling_brain_tumors.htm

University of Utah: Interactive

<http://learn.genetics.utah.edu/content/tech/stemcells/>

Molecular Movies: Stem cells, Breast Stem Cells, Control of Breast Stem Cells, Origin of Breast Cancer

<http://www.molecularmovies.com/showcase/#Stem%20Cells>

LBNL News Story:

<http://www.ascnetwork.com/component/content/article/52-february-2009/370-it-takes-a-village-cell-microenvironments-hold-key-to-future-cancer-and-regenerative-medicine-therapies.html>

LaBarge paper (Bissel Lab)

<http://www.rsc.org/ej/IB/2009/b816472j.pdf>

Cell cycle, Cell divisions, Cancer

<http://www.nature.com/scitable/topic/cell-cycle-and-cell-division-14122649>

AND

<http://www.nature.com/nature/journal/v411/n6835/abs/411375a0.html>